Environmental Health Perspectives
Vol. 27, pp. 197–206, 1978

Summary and Perspectives

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The job of attempting any sort of summary of so much material of such diversity is a formidable one, certainly when the task allocated includes sketching some perspectives. I shall attempt to tackle it in three stages, first mentioning some of the highlights from the various contributions, then offering a few general comments on characteristics of higher plants as test subjects for mutagen monitoring, and concluding with a brief—indeed very brief—consideration of perspectives. My comments are from one whose interests lie in plant physiology and cytogenetics; I am not myself active in the field of mutagenesis, so I hope you will bear with my deficiencies should anything I say sound erroneous or tendentious.

The workshop opened with the paper from Dr. de Serres, who offered us some targets for the meeting. He reminded us of the range of purposes for which we may wish to monitor the environment for mutagenic agents, noting that we are not only concerned with the well-being of man, but with his plants and animals—and one might hope also the biome at large, not only those elements of it of direct human concern. He mentioned the impressive figure of 63,000 man-made chemicals now littered about the earth, with a growth rate of some 3000 new ones per year: indeed an intimidating screening task. As for the screening systems, he stressed the need for specificity; the requirement that the data gathered should be relevant to the problem, a consideration of great importance when it is proposed to extrapolate from results obtained with one class of organism to another, different, class. Dr. Heath followed with a sobering talk in which he gave us a glimpse of cancer epidemiology in the U. S. Among the many significant points he made I recall especially his comment on the long latent period between first exposure to a causal agent and the ultimate effect. He referred to the case histories of kepone, polybrominated biphenyls, and vinyl chloride, important to have in the back of one's mind in thinking about the predictive powers of testing and monitoring procedures. Dr. Shelby reviewed for us the NIEHS-sponsored EMIC data system, and enumerated for us some of the current users. He noted that the data base was accessible through various agencies, but one wonders whether it is as yet widely enough known to the field of potential users. Dr. Shelby also referred to the need for chemical precision in referring to mutagenic and carcinogenetic compounds, stressing the importance not only of proper identification of the materials but of providing estimates of their purity. I might add that biologists, for their part, would do well to ensure that their material, also, is properly identified. This means something more than finding a convenient Latin binomial when it comes to the use of higher plants; there must be good taxonomic control in the identification of cultivars, and a proper specification of provenance when natural populations are used. Should it come to the setting up of a network of testing stations, it will be valuable to develop clonal sources, as in the Brookhaven *Tradescantia* programme.

Dr. Vig's paper provided us with some beautiful examples of somatic mutation in familiar plants, expressed in the flecking of leaves by the production of chlorophyll abnormalities. As Dr. Vig emphasized, the effects can arise from a diversity of mutational events, and the interpretations of phenotypic effects should ideally be tested by recovering plants from tissue sectors so that genetical analysis can be carried out. But even without this, the results obtained with known mutagens show that the system has potential for monitoring and so merits continued development.

Dr. Grant gave us a dramatic display of chromosomal effects of pesticides, ranging from those involving interpretable mechanical aberrations including breakages, deletions, inversions and translocations to mysterious defects involving stickiness, clumping, loss of basiphilia and despiralization. The

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biochemical basis of much of all this remains obscure, presenting a challenging field for future work. In considering Dr. Grant's contribution, one must bear in mind that pesticides are, after all, intended to upset the metabolism of plants and animals, so it is not surprising that they do disturb nuclear behavior. The question always is whether the damage to the target is sufficiently greater than that to the organisms we wish to conserve, including of course ourselves. Dr. Plewa also talked about the effects of pesticides, considering the capacity of *Zea mays* to convert certain classes of herbicides into mutagens. You will recall that he gave us a lucid exposition of the potential of pollen assay systems using the *waxy* locus, an example of exploiting the advantage the angiosperms offer of having a haploid generation with limited gene expression.

The tandem contributions of Dr. Van't Hof and Mr. Schairer dealt with the work based at Brookhaven using the *Tradescantia* staminal hair test system and the mobile laboratory developed with NIEHS funding. Their two presentations showed what can already be done with efficient higher-plant test systems using uniform genetic material—and of course with the backing of the appropriate resources. The test system no doubt requires further genetical study, but its effectiveness is clearly not in doubt. Its special advantages are surely the readiness with which scoring can be done and the way it can be used to detect low levels of mutagens in the atmosphere through its capacity to accumulate effects over protracted periods of time. The data obtained from the field surveys raised many intriguing questions, and it will be interesting to follow the progress of the attempts to determine which atmospheric contaminants actually are the mutagens in those sites where high activities were recorded.

Although Dr. Redei's contribution was not given as a formal paper, it was indeed a striking one, and I am sure we will all long carry in our memories the image of his *Arabidopsis* jungle. Obviously there is considerable potential in a species like this for mutagen monitoring, given the ease of cultivation in quantity and the fact that in an autogamous species highly homozygous pure-lines could be selected as testers.

Mr. Barnes dealt with higher plants as concentrators of environmental pollutants, and their use in coupled systems. Notwithstanding the difficulties he took care to stress, the basic scheme is an attractive one, bringing together as it can the capacity of plants in the field to accumulate mutagens and the testing precision given by laboratory assays, including the Ames test. Dr. Constantin recapitulated for us several of the potential advantages of higher plants for the direct testing of mutagenic activity, noting especially the value of loci with conspicuous and unambiguous phenotypic effects. He referred to the chlorophyll-deficient mutants of barley, and this recalled to my mind the fact that barley was one of the earliest plants to be used in research on chemical mutagenesis, in Gustafsson's laboratory now more than quarter of a century ago, at a time when the very concept of the chemical induction of mutations was novel. Dr. Freeling considered the potential value of one readily assayed gene complex, the alcohol dehydrogenase locus in maize. Since, like *waxy*, this is expressed in pollen, activity can be assessed in large populations of haploid plants allowing very precise analysis of mutation rates. He made a valuable point rather as an aside, namely that while studies of structural genes may be easier from a technical point of view because the problems of assaying are fewer, great importance also attaches to effects on regulatory genes. If we accept the idea that large parts of the eukaryotic genome constitute regulatory elements, then these form a major part of the target area; and of course it is in the regulatory systems that mutations producing developmental aberrations, including cancer, are likely to occur.

Dr. Chiscon described for us another "haploid" system, this time using cultured tobacco tissues—haploid in the sense that most cells have about half of the chromosome number of the parent plant, itself an allotetraploid. She referred to the problems of stability in cell cultures, but provided convincing evidence from the effects of known mutagens that such cultures can be used to detect and quantitate mutational events. Again it will be rewarding to develop this kind of system, using, as Dr. Chiscon suggested, true haploids, including those derived directly from pollen. Dr. Mulcahy's contribution dealt with the potential of gametophytic self-incompatibility systems for monitoring mutagenicity. Mutations in any part of the compound *S*-locus tend to give self-fertility and this is easily picked up by a straightforward screening system. He showed us how through a judicious choice of plants a field monitoring system could be set up requiring little attention, giving the facility for integrating effects over a period of time, and allowing simple analysis of results. It should obviously now be tested in a practical situation alongside others. My own choice for a comparative study would be the Brookhaven *Tradescantia* system.

Dr. Klekowski's contribution introduced us to the special world of the ferns, and gave an impressive demonstration of the various ways these plants can be pressed into service as environmental monitors. The mode of growth of the sporophyte in

the leptosporangiate species gives it the potential of accumulating genetic changes over long periods, and the fact that the gametophytes are free-living and autotrophic allows them to be used directly for the assay of post-zygotic mutations that affect growth and morphogenesis. One wonders about the potential of the other archegoniate groups with haploid dominated life cycles; there is clearly here an invitation to extend Dr. Klekowski's approach.

Dr. Epler introduced us to the complex chemistry of fuel processing, and showed us something of the procedures being used in screening by-products for mutagenicity and carcinogenicity. His talk emphasized the need for batteries of tests, and clearly showed how in such fields the collaboration of chemists and biologists is essential if the active compounds are to be identified and neutralized or excluded from the environment. One wonders how the research efforts of other industrial countries in this field match up to the perceptive studies now progressing at Oak Ridge. Dr. Ridgway's talk took up once again the matter of pesticides. He pointed out the intractable fact that, however much we may deplore it, the productivity of modern agriculture depends on large-scale pesticide usage. Maintenance of present populations and life-styles—willed by the majority, so it appears, in the industrialized countries—must therefore be bought at a certain cost of environmental pollution. The task to be faced is the evaluation of what the cost is now and what it is likely to be in the long term. In developing the theme of cost-benefit analysis, he brought very clearly to our attention the importance of good and comprehensive biological data, an absolute requirement for the work of regulatory agencies charged with the task of controlling pesticide usage. Dr. Menn, also on the theme of pesticides, discussed metabolic transformations and the implications of these both for activation and detoxification. He provided us with a comparison of plant and animal cells, and noted that higher plants, without excretory systems, tended to store degradation products. Since they then remain available for animals to consume there is a very real need to know what the end products are and to evaluate their biological activity. Dr. Cataldo made a similar point in his contribution on the accumulation of heavy metals by plants, indicating the special hazards that could arise when the storage is partly in the seed. His analysis of the uptake by roots of potentially toxic metals showed how the normal transport mechanisms could be perverted, so to speak, in polluted soils, when the plant lacks the power of discrimination between nutrient ions and others. The problem, long known in agriculture in relation to toxic elements in natural soils, could increase with the greater dispersal of man-released pollutants. Dr. Wolverton's entertaining paper on *Eichornia crassipes* showed how this aquatic species can be used as a concentrator of heavy metal contaminants in water, providing a valuable monitoring system. He also illustrated for us NASA-sponsored work on the exploitation of *Eichornia* for extracting water pollutants in water purification systems. The use could be as valuable on earth as in extra-terrestrial life support systems, and the field-size *Eichornia* purification column provided us with another visual memory to take away from the meeting.

Plants as monitors and concentrators of atmospheric pollutants were considered in the papers by Drs. Feder and Koranda. Dr. Feder's spectacular illustrations of ozone damage in certain tobacco cultivars showed how sensitive, specific, and economical assays based on the proper choice of plant test material can be. It is difficult to imagine any other means that could have demonstrated so convincingly the effects of ozone release from remote mainland sources on the concentration in the atmosphere of Nantucket Island, and as Dr. Feder pointed out, tobacco is not the only candidate for use in short-term atmospheric monitoring systems. The question here is one of monitoring pollutants causing immediate physiological damage and not of assaying for mutagenesis. But the different sensitivity of cultivars reminds us again how important it is going to be in selecting test systems to choose the appropriate genotypes and ensure that all users accumulate data for the same ones. Dr. Koranda surveyed the work on short- and long-term radionuclide accumulation in vegetation exposed to fall-out from atomic testing. He made the interesting distinction between foliar uptake in the short term, involving adsorption onto leaf surfaces or passage into the plant through the barrier of the leaf surface wax and the underlying cuticle, and the longer term uptake through the root system after entry of the radionuclides into the soil. One would have like to have heard more about the mutational load of vegetation in areas subject to intense fall-out, for the data could be valuable in assessing the potential of herbaceous and arboreal species as recorders of mutagenic episodes. He and Dr. Klekowski should get together!

In turning to my second theme, I am aware that much of what I shall say has already been touched upon, or indeed dealt with in depth, in Dr. Nilan's lecture; but perhaps my emphasis will be a little different. Also, many of my comments will sound like pleas for more botanical research—and that is how I intend it. It seems to me that before we can extract the maximum use from higher plants as

monitors of environmental hazards we do need considerably more information, and about a diversity of aspects. A leading question concerns the justification for supposing that we can extrapolate from higher plants to animals like ourselves. Undoubtedly the essential fact, brought out by Dr. Menn, is the high degree of similarity between plant and animal cells. Both are eukaryotic; both have the same mechanisms of DNA synthesis and, broadly speaking, of chromosome replication and karyokinesis; they have the same method of coding proteins; and, of course, they possess much of the basic cell metabolism in common. We suppose that they must share similar control systems in differentiation and development, obscure although these may be at this stage. Yet there comes a point where the differences become more important than the similarities, for after all plants are not animals. In our present context it may well be as important to be aware of the differences in superstructure, so to speak, as to know of the similarities in infrastructure. Even at the cellular level we cannot assume complete identity, and every generalization must be checked. In the discussion there was a reference to the diffuse centromere of *Luzula*, and this reminds us that even among the angiosperms themselves there is a good deal of variation in chromosome mechanics and the details of mitosis. This is true also of meiotic behavior. It is now known, for example, that the two monocotyledonous genera, *Lilium* and *Triticum*, differ in the events immediately preceding the meiotic prophase in such a way as to affect their responses to spindle poisons. Obviously, variation in the mechanics of chromosome association and movement could have considerable significance in explaining the diverse responses to mutagens affecting chromosome structure in different test systems.

Another dimension of variation that surely must be taken into account in comparing mutagen effects is the extraordinary range of DNA content in the nuclei of higher plants. It is true that if one takes a sequence of evolutionary grades, starting, say, with unicellular algae and working up to oak trees, there is a broad trend of increasing DNA content per haploid chromosome set, but within a single evolutionary grade like the angiosperms there is enormous variation, even discounting polyploidy. The disparity between some potential test organisms, and between them and man is very great. Certain lily species have more than 100 pg DNA per chromosome set, 50 times that of *Arabidopsis* and an order of magnitude greater than man. We still have no real idea of what all the DNA in some plants is all about. It may indeed by largely redundant—carried as a useless metabolic load much of which could be fined away without affecting the function of structural genes or the systems that regulate them. But can we say that it has no significance in relation to the effects of mutagens? It would be good to have some answers in this field.

Moving outside of the nucleus we come to the arena of the cytoplasm, often overlooked in mutation research. In plants there are three genomes in the cell: that in the nucleus and those of the mitochondria and plastids. All interact and show mutual regulatory effects. The presence of an additional genetic system in the plastids certainly accounts for some of the differences between plants and animals, and it can scarcely be ignored in devising test systems. Mutation of the plastid genome can produce somatic mosaicism, but it is not always apparent how this might be expressed. During the progression from the cells of the meristem to the final differentiation in the palisade tissue of the leaf there are several cycles of plastid division. Each involves replication of the plastid DNA and a corresponding increase of the number of copies of the plastid genome, all available as mutagen targets. Whether mutations would be phenotypically detectable would depend on a number of circumstances: when in the lineage the mutation took place, whether there was a sorting out of the plastid genomes of the cytoplasmic heterozygotes during cell division, and whether the defects affected the viability of the cells. On the whole one does not suppose that mutations in the cytoplasmic genomes would be expressed very readily, so there is good reason to accept that the leaf pigment mutants studied by Dr. Vig will prove to be nuclear. But it would be as well to know a little more about the effects of environmental mutagens on the plastid genomes of higher plants, the cytoplasmic genetics of which lags well behind that of *Chlamydomonas* in depth of biochemical understanding.

Plant and animal cell protoplasts differ also in the presence of vacuoles as a characteristic feature of the former. The vacuole forms part of the osmoregulatory system of the cell; but it is far more than that. In some plants it is a kind of lyzosome. Arising from an embayment of the endoplasmic reticulum, it may acquire an enzyme content and establish thereby a special metabolic compartment of the cell. It may also serve as a kind of metabolic "dump," receiving products from the cytoplasm and storing them out of the way, so to speak. In such a role it can do for the plant cell what excretion does for the animal, namely provide a waste disposal system for handling undesirable products. This storage of toxic materials, including metal contaminants taken up from the atmosphere or soil, solves a problem for the plant, but leaves one for

the animal that may subsequently eat it. Plants as concentrators and processors of environmental contaminants were considered in Mr. Barnes's paper, and we have had some discussion of their possible function as mutagen activators. In this connection it is worth reminding ourselves that animal cell technology often cannot be applied to plant cells with the expectation of comparable results. We may take the question of the so-called "microsome" fraction, mentioned in connection with *in vitro* activation systems. The microsome is not a biological structure: not an organelle. It is a biochemical object, created when a cell is processed in a certain way. Microsomes are obtained when the cells of pancreas or liver are homogenized; the endoplasmic reticulum is broken into minute, ribosome-studded vesicles; each a microsome in the biochemical sense, retaining its functional identity because the enzymes are held in the membranes. Such fractions are unlikely to be yielded by many mature plant tissues because these often do not have a copious endoplasmic reticulum, lacking as they do the special synthetic and secretory functions of animal gland tissues. Indeed, it is sometimes very difficult to find any endoplasmic reticulum at all in vacuolated storage tissues in which cells have reached their terminal differentiation. To grind up leaves, say, in the hope of getting fractions with metabolic activity comparable with that of similar preparations from massive animal tissues is to seek something that cannot be achieved. This is not to say that there are no plant tissues that could be used in standard ways to obtain activating fractions; but they will have to be looked for. I do not think many plant cell biologists will have had reason as yet to consider this point, but if it is thought necessary to see what plant cell fractions can do to environmental contaminants suspected as mutagen precursors it should not be too difficult to develop test systems.

In considering the cell in relation to its environment we meet yet another major difference between the two great eukaryotic groups: the possession by most plant cells of a wall of greater or lesser rigidity. Animal cells have carbohydrate-rich surface coats, contributed to by the heterosaccharide parts of membrane glycoproteins and glycolipids. The plasmalemma of the plant cell appears to have a similar organization, and no doubt the integral membrane glycoproteins and glycolipids have terminal sugars outside of the cell. But in addition there is the wall: the box within which the cell lives. It has been suggested that the wall can be homologized with the glycocalyx of the animal cell, but I do not think this is a useful extrapolation. The plant cell wall is a structural entity made up of cellulose microfibrils and matrix materials including hemicelluloses, pectic substances, and, as we are now beginning to learn, considerable amounts of protein, including glycoproteins. In some cell types, the walls contain lectins and enzymes, synthesized in the cortical layers of the cytoplasma and passed out through the plasmalemma. The whole system of walls in a plant tissue forms a potential pathway for the movement of materials, the extracellular or apoplastic pathway. An important characteristic is that it is most effective for the movement by diffusion of water-soluble compounds; it is not an easy channel for the passage of hydrophobic compounds. Because there is no direct access to the plasmalemma, neighboring cells can never achieve the intimate contacts found in animal tissues. Within plant tissues communication between protoplasts is through the plasmodesmata, this forming the so-called symplastic route. There may be direct channels of communication to the plant surface, ectodesmata, but little is known about these, and even their very existence is open to doubt. The outer surface of the aerial parts is in fact very well protected, as Dr. Koranda reminded us this afternoon. The pectocellulosic outer walls of the leaf cells bear a cuticle, and this in turn is often overlaid with wax. The product is a sandwich of hydrophilic and hydrophobic materials: not an easy one for any compound to cross. Yet of course some do, and it would be a worthwhile exercise to find out how selective this composite barrier is in the uptake of potential mutagens through aerial parts and how effectively the compounds taken up are distributed in the tissues in assessing the utility of higher plants as test systems.

The situation is different for roots. Their function is to take up materials from the soil environment, and this they do with great efficiency, but not necessarily very selectively, as Dr. Cataldo's paper indicated. Roots have but limited powers of discrimination, and it is not easy to predict *a priori* how they might handle unfamiliar materials encountered in the soil solution. The main function of roots is to concentrate ions from weak solutions; the pumps responsible for this have evolved to meet the nutritional needs of the plants, not to sort out man-made chemicals presented to them in polluted environments, let alone to act as concentrators for the convenience of mutagen test systems. Many soluble compounds from the soil are likely to enter directly into the root free-space, but once there they must pass the barrier of the endodermis before being loaded into the transport system and moved into the aerial parts of the plant. Recent work on the function of the endodermis shows that older ideas that it acts as a barrier to the passage of soluble materials through the apoplastic channel and en-

forces entry into the symplastic route are indeed correct. But little is known of the function of the endodermis as a screening and processing layer; indeed, little is known about any aspect of its metabolism, except that the cells during their differentiation deposit lipidic materials in the wall to form the Casparian strip. It may play no other part than that of funnelling materials into the symplasm before the loading of the translocation system; or it may be involved in metabolic conversions between the point of entry at the soil-root interface and the shoot system. The topic would merit study.

In the course of our discussions there has been repeated reference to the need for more information about certain aspects of plant growth and development in the light of the special requirements mutagen testing might impose. Perhaps the most important feature to bear in mind in this context is the characteristic mode of growth of higher plants, through the agency of apical meristems. Unlike the higher animal, the plant is perpetually embryonic. It does not have a definable early period of embryogenesis during which all of the principal organs are blocked out preparatory to a period of growth, but is continuously involved in organogenesis. Accordingly, it is liable to birth defects, so to speak, at any time when still alive. This developmental pattern can be of great value considered from the point of view of environmental monitoring. As Dr. Klekowski brought out, the leptosporangiate ferns with their simple apical organization have axes that are essentially clones of cells originating from a single apical cell. The situation is different in gymnosperms and angiosperms, where the meristem is a massive structure. The population of cells in the meristem does not of course increase exponentially; rather is it the case that certain zones are composed of cells the primary function of which is to divide. On the average one of the daughters of each division differentiates or gives rise to a lineage destined to contribute to the soma while the other continues the meristematic function. There is therefore a finite population of cells that continuously produces the plant body, a population which as a whole is the functional equivalent of the apical cell of the fern.

Certain consequences arise from this. We see, for example, a further distinction from the higher animal: there is no germ-line, no volume of cells destined to produce the gonads in the due course of development. Any of the lineages originating in the meristem, except those producing the dermal tissues, as well as contributing somatic tissues could be the progenitors of the generative cells. Much work has been done on meristem organization, both in this country and in Europe, and there is a substantial literature on the topic; but we still lack a full understanding of the dynamics of the apex, certainly for many of the plants mentioned in this meeting. For example, there is inadequate information about the fates of cell lineages. Our experience with the effects on apical organization of base analogs a few years ago convinced me that there is much competition between lineages, and a form of intrameristem selection is the consequence, with defective lineages being eliminated. Compare this with the fern apex: here, unless the apical cell suffers a drastic accident that renders it inviable or incapable of division it will continue to produce shoot tissues, handing on any genetic defects. In the massive meristem of the angiosperm even a slightly defective lineage runs the risk of being squeezed out in competition with others. The apex has, therefore, a kind of continuously active repair system, operating to compensate for genetic lesions—the probable reason, indeed, for the success of this type of organization in evolution. But how effective is this mechanism? There is need now for a fuller study of intrameristem competition using labeling techniques, microsurgical methods including lasers, and possibly employing genetical chimaeras, much studied in pre-war years in relation to histogenesis. Without a fuller understanding of these aspects of meristem function I do not think we can expect to make full use of growing shoot systems as potential registers of genetic damage.

These considerations could well affect our selection of plants as test organisms. We have already considered the special qualities of ferns for the purpose; but one must admit that among flowering plants the choice hitherto has been largely fortuitous. Undoubtedly the rationale is clear enough for *Arabidopsis*, with its extraordinary capacity for rapid growth and reproduction in controlled environments, and *Tradescantia*, with its convenient and accessible staminal hairs, chosen so perceptively by the late Dr. Arnold Sparrow. For the rest, there has been a natural inclination to use crop plants because so much is known about them genetically and physiologically, and because they are readily available in standard genotypes and are readily cultivated. But they were designed in the first instance for very different purposes, and it does not follow that they are well adapted for environmental monitoring. It may be necessary now to draw up a blueprint for a tester, and if a plant is not available to meet the requirement, to breed one for the purpose.

This is not such a far-out suggestion as it may seem, given that we can draw up a list of criteria against which to match the candidates. For example, if we wish to monitor chromosome effects, and particularly if we hope to put the work of scoring

into the hands of staff with no special cytological training, a low chromosome number would be advantageous to facilitate both mitotic and meiotic studies. *Crepis capillaris* has been mentioned; perhaps even more favourable from this point of view would be *Haplopappus gracilis*, with two chromosomes per set. There are other desiderata, including the ease with which plants can be regenerated from cultured cells and tissues. Rapid generation time, ease of cultivation and ready availability would all be pertinent factors if it were eventually judged necessary to set up a special monitoring system using standard test objects at different sites.

There are many opportunities for using wild plant populations as monitors, the ferns providing the best examples to date. Clonally spreading flowering plants might also be considered. Many examples come to mind: one is the clonal populations of aspens, seen around lakes in northern USA and Canada. Do such clonal populations register and store mutational events? Do they show sectoring, like a *Neurospora* plate? Or are the stabilizing mechanisms operating in the meristems effective in maintaining the norm over hundreds or even thousands of years? The same questions might be posed for many herbaceous species. *Paris quadrifolia*, a clonally spreading species of the ground flora of forests, is known to have a fairly simple karyotype and to show an astonishing amount of cytological variation in inversions and translocations. Do the clones preserve records of past chromosomal mutation, and might it be possible to construct time records of these? If so, they could provide useful cross-checks for records obtained from fern populations and other sources.

A recurrent theme in the deliberations of this meeting has been the problem of distinguishing different kinds of induced change. I have touched upon the difficulties of making a distinction between organelle and nuclear mutations, and we might also note that with higher plants it is often scarcely possible to discriminate between genetic and epigenetic effects if we are constrained to inspect phenotypes. If we are to make these various distinctions, it will nearly always be necessary to take the plants through one or more cycle of sexual reproduction. It may be argued, however, that for some purposes we do not need to know the precise basis for induced aberrations anyway. One could use the epigenetic changes themselves as another index of harmful environmental effects if they represent persistent departures from the norm of development. Such an index, could it be reliably calibrated, might even have special value in respect to carcinogenicity. But we lack adequate insight into the control mechanisms in both plant and animal differentiation to be able to make such a claim; and it is more likely that at this level the developmental physiology of plants and animals is sufficiently dissimilar to make any extrapolation suspect. However, there is a considerable botanical literature relating to induced development aberrations. Some involve no more than transient teratism (we may recall here my comment on the proneness of the plant to birth defects at any age), while others are persistent even through cycles of sexual reproduction. Examples of the latter induced by simple agents such as chloral hydrate were documented in the 1920s and 1930s under the name of dauermodifications. These defects are not genetic, although inherited in a limited degree through the female lineage. There has been no recent work on dauermodifications—certainly none from the standpoint of molecular biology—leaving one more topic requiring re-investigation.

The further analysis of somatic mutations, particularly those affecting limited sectors of the plant, requires that by some means the tissues concerned should be made to regenerate reproductive structures so that segregation and recombination can be followed. This is true also for monitoring systems in which cultured tissue are themselves used as the test object. At the present stage of plant tissue and cell culture technology it is not possible to guarantee that the regeneration of complete plants can always be achieved: in some families, yes—as with the Umbelliferae; in others, including important economic families like the Gramineae, the job is difficult. The criterion of regenerative capacity therefore becomes an important one also for the choice of tissue sources for *in vitro* test systems.

Such systems present special problems of their own, one being a tendency towards instability. It is rarely found that regeneration can be obtained from long-term cultures, even in the case of the amenable carrot plant, probably because the surviving cells in long term cultures are those selected for life *in vitro*—cells that may have lost entirely the competence for organogenesis. But there is another problem. Cultured plant tissues are well known to accumulate aberrant karyotypes, with variation both in chromosome number and structure, and they tend also to store gene mutations in a way never seen in the intact plant. I believe myself that much of this arises because the selective pressures acting between cell lineages in the meristems and subadjacent tissues are released *in vitro*, allowing a free rein to mutations, both chromosomal and genic, that would never be given during integrated growth where the social behavior of cells is much more strictly regulated than in freely-dividing callus.

But this leads to another intriguing question: to

what extent is the instability of cells in cultured tissues itself an expression of mutagenic influences? Even if there is a high baseline of "spontaneous" aberration in such systems, variations above this baseline might, appropriately calibrated, provide a useful index of mutagen action. So far as I am aware little consideration has been given to this matter until the work reported by Dr. Chiscon.

I would like to turn now to haploid systems using pollen, mentioned in several papers and during the discussion. Pollen, of course, represents one sex of the alternate generation in the angiosperm life cycle; each pollen grain is a haploid plant, not a gamete; and each has its own metabolism and independent gene expression. It is also true, however, that the pollen plant inherits some of its metabolism from the parent meiocyte, and the number of genes actually transcribed in the haploid generation is probably quite small. We have heard some dramatic examples of the use of genes that are active in the haploid phase in mutagen monitoring systems—the *waxy* locus in Dr. Plewa's paper and the *alcohol dehydrogenase* locus in Dr. Freeling's—and Dr. Mulcahy has sketched for us the potential of the incompatibility locus. This does not exhaust the range of possibilities. Some years ago Haldane noted that the haploid generation provided something of a test for the genome to the extent that lethal mutations, even when recessive, would necessarily be screened out were they expressed in that generation. Pollen viability itself can therefore provide an assay, just as the viability and developmental behaviour of fern gametophytes.

It seems clear, however, that because much of the genome is silent in the gametophytes lethals effective in the sporophyte are transmitted readily enough. But we do not yet know just how much expression there might be in pollen and embryo sacs—whether, say, there are marginal effects in the gametophytes of genes that find fuller expression in the sporophyte. Dr. Mulcahy has pointed out to me that it might be quite important to find out, for example, whether the heavy metal tolerances of pollen tubes are correlated with those of the parent. Is the vegetative cell in such a case acting as though it were simply a detached fragment of parent cytoplasm, or are the genes concerned with metal tolerance also active in the gametophyte? If so, there may be still other specific loci for which pollen-based tests could be set up in mutagen monitoring systems.

What, then, can one say about the longer-term perspectives? I suppose all of us active in this field came to this workshop meeting with a bias; we came already with the conviction that plant systems could be used in the monitoring of environmental mutagens, and we were already convinced that at least there should be some further testing of their potential. Some of us no doubt came with the hope of gaining some assurance that our work is valid and valuable, and I think the meeting will have done much to establish this. However, participants in this meeting are not concerned only to convince each other, but to present what they have to offer to a wider market—to other biologists, and to the greater world of those using biological information in industry, medicine and government. Viewing the matter as dispassionately as one can, I think it is fair to conclude from the proceedings of this meeting that plant systems can often provide a useful supplement to other mutagen monitoring systems available, and can sometimes do more. And if that is so, there is obviously an obligation to develop such systems and show how they can be used in practice.

One could take the view that this workshop is concerned wholly with techniques—ways of doing things—and that those attending it are simply high-grade technicians. This is rather a popular view with administrators, who are inclined to think that scientists are bright enough to get the data and make the predictions but not bright enough to have anything to do with policy. I suppose none of us here would subscribe to this; and it would indeed be a dereliction of duty not to offer some opinions on policy. It is difficult in discussing this not to get into political, sociological, and moral considerations; moreover, one's thoughts on this topic must necessarily embrace the whole matter of mutagen monitoring, not simply the application of higher plant systems. I must, however, restrain myself at this late hour from the temptation of raising more than a couple of matters. Firstly, it seems to me abundantly clear that we already know enough about the hazards of environmental mutagens to assure us that we need to know more. We cannot have too much evidence, and all sources must be tapped. Nothing should be rejected *a priori* because we do not know yet what it has to yield; until the overall picture is much clearer, information must simply be accumulated from wherever it is available. We are all very well aware of how slender the hard biological knowledge of the nature and extent of the hazard of environmental mutagens actually is; and we know at the same time how hungry for data regulatory agencies and others are. Economic and political modelling are popular activities today: every government department models something or the other, as does every government laboratory. The worry we all

must have is that the models, no matter how strong they may be mathematically, can have no greater validity than the biological data on which they are based. The credibility that can be given to garbage by computer processing is one of the more frightening features of the modern scene. If we wish to be reminded of how important it is to have good basic data, we might recall Dr. Ridgway's comments on cost-benefit analysis in pesticide usage. One wonders just how often huge superstructures are founded upon little snippets of data—perhaps no more than hunches—which any biologist would hesitate to include in a research paper were it likely to be exposed to peer review.

The second point arises from the first. Even with an adequate base of data on which to judge the current scene and to assess what has happened in the past, prediction of what might happen next is always going to be immensely difficult, and that fact itself must be taken into consideration in relation to policy. Economic modeling systems tend to work on the concept of really very short feed-back loops. Market forces and current performance are assessed, and their impact predicted in terms of sales and profitability so that production, design and other strategies can be adjusted to achieve certain aims. Five or ten years is a long time for predictive economic modeling, yet we all know perfectly well that in the field we are considering in this meeting the time constants are very much longer. As Dr. Heath's paper grimly reminded us, the latent period for environmentally-induced cancers is not a matter of five or ten years, but twenty or thirty. With mutagens, it could be generations. I do not really see how modeling can help us here when there are so many imponderables. Certainly any idea of cost-benefit analysis is absurd, for those who gain the benefits are not necessarily those who bear the costs. We enjoy the benefits of industrial production; our progeny suffer from the benefits we have taken. This is all perhaps so obvious to the present audience that it does not need to be said; but the issue is a vital one, and must influence the ways we go about presenting our information and addressing those who are going to use it.

What then is to be done in the follow up to this workshop meeting? Many of the contributions, and much of what I have said myself, have underlined the need for more research. A problem here is going to be to induce competent people to undertake it. I cannot see that there is as yet much awareness of the needs, at least among the bulk of plant scientists. The granting agencies certainly have a role here. Given the readiness of the scientific community to participate vigorously in the business of determining research priorities, there seems no reason why there should not be a conscious effort to encourage the development of grant proposals in the appropriate fields. Persuasion of this nature—powerful because of its relationship to funding—need not menace academic freedom nor scientific purity. After all, there are huge areas of basic research related to the topics we have been discussing all awaiting attention, and many would surely give as great a satisfaction to the creative scientist as would work on something without relevance to man's life on earth.

In the course of the discussions, and particularly after Dr. Nilan's lecture, we gave some consideration to the means of bringing the matter of this workshop to the attention of the biological community at large. We need, of course, to decide just how much prominence it requires. But if there seems good reason for extending the field of awareness, mechanisms are available. Motions can be proposed and discussed at the international congresses. The International Union of Biological Sciences and its Divisions, each with links to the national academies, provides another channel. Some attention also needs to be given to the part the international agencies might play. Most of what we have heard in this meeting has been related to activities in the U. S., and properly so, since in this field as in so many others the U. S. is making the pace. But the problem is certainly not a national one, not even for a country as large as the U. S. It is one for the world community, and this means in the first instance UNESCO, WHO, and UNEP (United Nations Environment Programme). The latter agency is developing an environmental data system, and the mutagen data base discussed by Dr. Shelby has been considered an integral part of this data system.

This leads me to my final comment, arising from the question: should someone be thinking about the possibilities of international monitoring systems using higher plants? Obviously the answer must depend upon an assessment of the severity of the problem overall and the effectiveness of other available systems. I am myself in no position to offer an opinion. But what I do assert, however, is that if a system is needed in one country, then we can be sure that in view of the disseminated nature of the hazards it will be valuable in others as a means of accumulating data to provide the basis for international agreements on control. Perhaps we can benefit here from the circumstance that the developed countries of the world, responsible for most of the hazards in the first place, are likely to become

most quickly aware of the problems and to have the greatest competence for dealing with them. There is an interesting contrast with the problems of conservation: these are mostly in developing countries, while the awareness of them and the resources to deal with them are mostly in the developed countries—a situation full of political difficulties.

It remains now only for me to thank those who conceived and planned this meeting, and to congratulate them on the success of their work—Dr. de Serres and the Program Committee, and NIEHS as the agency that made it possible. The meeting has been immensely informative—I think for all of us, but more particularly for those, like myself, who came to it with little prior knowledge of the field. All of us will go home with widened horizons; and those actively engaged in research on mutagenesis, with renewed dedication.